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Divide and conquer: Identifying Acute Respiratory Distress Syndrome sub-phenotypes

Manu Shankar-Hari[1,2] and Daniel F. McAuley[3,4#]

[1]Department of Critical Care Medicine, Guy's and St Thomas' NHS Foundation Trust, Westminster Bridge Rd, London SE17EH, United Kingdom; [2]Division of Infection and Immunity, King's College London, United Kingdom; [3]Centre for Experimental Medicine, Wellcome-Wolfson Institute for Experimental Medicine, Queen's University of Belfast, Belfast, Northern Ireland; [4]Regional Intensive Care Unit, Royal Victoria Hospital, Grosvenor Road, Belfast, Northern Ireland

Manu Shankar-Hari[1,2] M.Sc., Ph.D.

email: manu.shankar-hari@kcl.ac.uk

Twitter: @msh_manu

[#]Corresponding author

Professor Daniel F McAuley

[3]Centre for Experimental Medicine, Wellcome-Wolfson Institute for Experimental Medicine, Queen's University of Belfast, Belfast, Northern Ireland; [4]Regional Intensive Care Unit, Royal Victoria Hospital, Grosvenor Road, Belfast, Northern Ireland

email: d.f.mcauley@qub.ac.uk

Tel: 0044 28 90635794

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The acute respiratory distress syndrome (ARDS) definition identifies patients with acute onset hypoxaemia and respiratory failure, who have bilateral opacities on chest radiograph that are not fully explained by cardiac failure or fluid overload[1]. ARDS is a common illness that accounts for approximately 10% of critical care admissions and 20% of patients requiring mechanical ventilation[2]. The hospital mortality in patients with ARDS remains high, increasing from approximately 35% for those with mild disease to 46% for those with severe ARDS[2]. This high mortality has remained relatively unchanged in the last 20 years[3]. To date, despite decades of research, there is no pharmacological treatment that can modify the underlying biological mechanisms implicated in ARDS and improve patient outcomes[4]. Within ARDS populations there is substantial biological and outcome heterogeneity, with observed differences in dominant pathogenic mechanisms, treatment responses and outcomes[5-7]. Identifying ARDS sub-phenotypes based on biological characteristics mechanistically linked to specific therapies irrespective of the baseline risk of outcome, is the conceptual definition of predictive enrichment[7 8]. The identification of such ARDS sub-phenotypes will enable improved trial design in ARDS by selecting patients based on responder characteristics to therapeutic interventions, hopefully resulting in improved outcomes[6].

In this issue of Thorax, Bos et al report an excellent cohort study in 700 ARDS patients, testing the hypothesis that ARDS sub-groups exist due to difference in biological characteristics[9]. In this retrospective analysis of a prospectively collected cohort, 20 biomarkers were selected to represent inflammation, coagulation and endothelial activation, as hallmarks of ARDS biology[6]. The dataset was divided into a training cohort (n=454 patients) and validation cohort (n=246 patients), based on the study recruitment period. Cluster analysis was used to identify homogenous ARDS sub-phenotypes in the training cohort[10]. The most predictive biomarkers were then confirmed in the validation cohort. These biological clusters were then linked to clinical and outcome characteristics of ARDS patients to derive clinical sub-phenotypes, namely *reactive* and *uninflamed*. These two clinical ARDS sub-phenotypes differed in terms of illness severity and critical care mortality, with the reactive group having a greater risk of death.

A key question for the reader is whether these associations are spurious or indirect or causal?[11] Cluster analysis methods generate different results dependent on the variables chosen for identifying similarities between patients and the method of clustering[10]. Bos et al chose biomarker characteristics as the variables on which the groups should be similar, and used Ward's method of

agglomerative hierarchical clustering to identify two potentially generalizable ARDS clusters. Hierarchical clustering is a commonly used iterative method to identify homogenous groups or clusters based on specific characteristics. In the paper by Bos et al, the goal was to identify ARDS patients with similar biomarker profiles, from a heterogeneous ARDS cohort. The basic algorithm starts with assigning each ARDS patient a 'value' based on their individual biomarker profile. Then patients with similar 'values' are grouped together to form clusters. The underlying principle is that ARDS patients *within* each cluster will have similar biomarker profiles and that *between* clusters biomarker profiles will be different. Depending on the parameters specified, the same dataset can result in potentially different results with different clustering algorithms and there are no universally agreed optimal rule(s) for clustering[10]. Another potential limitation is that only patients with data on all chosen biomarkers were included and missing data in clinical variables were imputed, which has the potential for selection and information bias. The blood sampling window for biomarker measurement in this cohort was wide and drawn either on the day of ARDS diagnosis or the day before or the day after, challenging the time-based arguments for a causal relationship. Despite these challenges, Bos et al provide important data with strong associations that are consistent with our current knowledge, have biological plausibility and external validity.

Calfee and colleagues have led the field in defining ARDS sub-phenotypes. Using latent class analysis (LCA) of clinical and biomarker data from patients enrolled in ARDS randomised controlled trials, Calfee *et al* have originally identified two ARDS sub-phenotypes[12 13]. The *reactive* sub-phenotype identified in this study shares many of the features of the *hyperinflammatory* ARDS sub-phenotype reported previously[12 13], although the proportion of patients in the *reactive* group is much higher than the *hyperinflammatory* sub-phenotype. This suggests that the *hyperinflammatory and reactive* groups may represent a similar sub-phenotype, although this is unproven. The findings from this study are significant in that they have identified comparable sub-phenotypes in an observational cohort of patients with ARDS using a different analytic approach. Whilst Calfee et al identified these ARDS sub phenotypes using clinical and biomarker data, Bos et al identified them purely on biomarker data. It would be important to test whether similar sub-phenotypes emerge after harmonising these different study datasets and performing both cluster and latent class analyses. Table 1 provides a comparative summary of these three studies.

Table 1: Summary of studies that report ARDS sub-phenotypes

Parameter	Bos et al[9]	Calfee C et al[13]	Famous et al[12]
Sample size	700	1022	1000
Recruitment period	2011 – 2013	1996 – 2002	2000 - 2005
Study design	Observational cohort	RCT analysed as cohort	RCT analysed as cohort
ARDS P/F criteria	<=300	<300	<300
Blood sampling	Around ARDS diagnosis	At baseline	At baseline
Biomarkers used for deriving sub-phenotypes	**Lung epithelial**: *none* **Endothelial**: E-selectin; P-selectin; ANG1/2 **Coagulation**: Antithrombin; D-Dimer; tPA; PAI-1; **Inflammation:** Fractalkine; GM-CSF; ICAM-1; IFN-γ; IL-1β; IL-6; IL-8; IL-10; IL-13; TNF-α; MMP-8; TIMP-1;	**Lung epithelial:** SP-D **Endothelial:** ICAM-1; vWF **Coagulation:** Protein C; PAI-1 **Inflammation:** sTNFR-1; IL-6; IL-8	**Lung epithelial:** SP-D **Endothelial:** ICAM-1; vWF **Coagulation:** Protein C; PAI-1 **Inflammation:** sTNFR-1; IL-6; IL-8
Clinical variables used for deriving sub-phenotypes	None	Age, gender, ethnicity, BMI, Respiratory[#]; Cardiovascular*; Creatinine; Urine output; Bilirubin; Temperature; Haematocrit; WBC count; Sodium; glucose; Albumin; Platelets; bicarbonate; Aetiology of ARDS[^]	Age, gender, ethnicity, BMI, Respiratory[#]; Cardiovascular*; Creatinine; Urine output; Bilirubin; Temperature; Haematocrit; WBC count; Sodium; glucose; Albumin; Platelets; bicarbonate; Aetiology of ARDS[^]
Analytical approach to derive ARDS subsets	Cluster analyses based only on biomarker data	Latent class analyses based grouping based on clinical and biomarker data	Latent class analyses based grouping based on clinical and biomarker data
ARDS subset (prevalence %)	Reactive phenotype (58.0%) Vs Uninflamed (42.0%)	Hyper-Inflammatory (29.4%) Vs Phenotype 1 (70.6%)	Hyper-Inflammatory (27.3%) Vs Phenotype 1 (72.7%)
Mortality (%) by ARDS subset	Reactive phenotype = 36.8% Vs Uninflamed = 14.9%	Hyper-Inflammatory = 47.3% Vs Phenotype 1 = 19.4%	Hyper-Inflammatory = 45.0% Vs Phenotype 1 = 22.0%
Discriminant markers between phenotypes	IL-6; IFN-gamma; ANG1/2; PAI-1	IL-6; sTNFR1; Vasopressor use; IL-8; HCO3	IL-8; sTNFR1; Vasopressor use; HCO3; minute ventilation

Legend to Table-1:

Table-1 shows the summary of three recent studies that report ARDS sub-phenotypes. The ***Respiratory system variables[#]*** included *minute ventilation, mean airway pressure, plateau pressure, respiratory rate, tidal volume, positive end-expiratory pressure; partial pressure of carbondioxide ($PaCO_2$) and PaO2/FiO2 ratio. The* ***Cardiovascular* system*** *variables include highest heart rate, lowest systolic blood pressure and vasopressor use. The* ***aetiology of ARDS[^]*** *was coded as Trauma, Sepsis, Aspiration, Pneumonia or Other.* ***Abbreviations:*** *P/F = PaO_2/FiO_2 ratio; ANG1/2 = Angiopoietin 1 and 2; tPA = Tissue plasminogen activator; PAI-1 = plasminogen activator inhibitor-1; GM-CSF = granulocyte-monocyte colony stimulating factor; ICAM-1 = intracellular adhesion molecule-1; IFN-γ = Interferon-gamma; IL-1β = interleukin-1 beta; Interleukins = IL-6; IL-8; IL-10; IL-13; TNF-α = Tumor necrosis factor-alpha; MMP-8 = matrix metalloproteinase-8; TIMP-1= tissue inhibitor of metalloproteinase-1; SP-D = Surfactant protein-D; vWF = von-Williebrand's Factor; sTNFR-1 = soluble Tumor necrosis factor receptor-1; BMI = body mass index; WBC = white blood cell count;*

Several important questions remain unanswered. First, assuming the *hyperinflammatory/reactive* sub-phenotype represents a common sub-phenotype, further work is needed to identify the key discriminant makers to reliably define this ARDS subset. Ideally a minimal data-set of variables could be identified to efficiently achieve this. Second, although it remains unknown if ARDS sub-phenotypes respond differently to pharmacotherapies, an important aspect of in developing pharmacotherapies targeted at the *hyperinflammatory/reactive* sub-phenotype will be to determine the stability of the ARDS subgroup over time. This is important to determine the therapeutic window to intervene with a therapy targeted at this sub-phenotype. In addition, it would be important to define if and how moving from this sub-phenotype to an *uninflamed* phenotype represents therapeutic success or failure to guide ongoing treatment. Third, development of point-of-care assays along with algorithms to define these ARDS sub-phenoytpes at the bedside in real-time is essential to enable this information to inform clinical trials targeting these sub-phenotypes.

In summary, ARDS continues to be a clinical and research challenge in terms of developing pharmacological therapies. Bos et al provide intriguing data that highlights the need for further work to identify ARDS subsets with defined treatable traits. These sub-phenotypes should be based on modifiable biological characteristics linked to both the risk of poor outcomes and response to the tested treatment. This will enable personalised care of patients with ARDS.

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